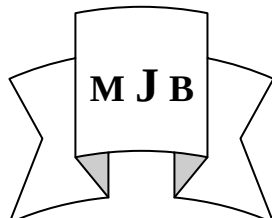


Study the Prevalence of ACL, APL, CMV, HSV, Rubella and Toxoplasma Gondii in Aborted Women in Baghdad

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Abstract

A total of 210 serum samples from women in Baghdad with history of one or more unexplained abortion were screened for the presence IgM and IgG antibodies against *Toxoplasma gondii*, *Cytomegalovirus*, *Herpes simplex virus II*, *Rubella virus*, anticardiolipin (ACL) and antiphosphatidylserine (APS) by using Enzyme-linked Immunosorbent assay (ELISA).

out of 210 aborted women in Baghdad a total of 44 (21%) were positive for IgM CMV Toxoplasma IgM positive were 32 (15.23%), Rubella IgM positive were 15 (7.14%) and 10 (4.76%) were positive for HSV II IgM also 12 (5.7%) were positive ACL IgG antibodies and 7 (3.3%) were positive for APS IgG and 7 aborted women showed mixed infection with CMV IgM antibodies, 5 were with Toxoplasma IgM, two with Rubella IgM. Moreover; the rate of one miscarriage in abortive women was 71.6% (86/120) higher than two and three miscarriages 25% (30/120), 3.4% (4/120) respectively with significant difference ($P < 0.05$). Also The results pointed out that the seropositivity rate for CMV IgM, *T. gondii* IgM, *Rubella* IgM, *HSV II* IgM, ACL IgG and APS IgG were higher in first trimester abortion when compared with second and third trimesters abortion with highly significant difference ($P < 0.05$).

الخلاصة

شملت الدراسة ٢١٠ عينة مصل دم لنساء مجهضات في مدينة بغداد للتحري عن وجود أجسام مضادة لكل من الكارديوليبين، الفوسفاتيديل سيرين، الفيروس المضخم للخلايا، فيروس الحصبة الألمانية، فيروس الهريس البسيط الثاني، وطفيلي المقوسات الكونيدية باستخدام تقنية الامتزاز المناعي المرتبط بالانزيم ELISA كما شملت الدراسة ٤٥ امرأة طبعية الحمل كمجموعة سيطرة .

أظهرت النتائج إن ٤٤ (٢١%) من النساء المجهضات كان لديهن الأجسام المناعية المضادة نوع IgM ضد الفيروس المضخم للخلايا و ٣٢ (١٥,٢٣%) كان لديهن الأجسام المناعية المضادة نوع IgM ضد طفيلي المقوسات الكونيدية، ١٥ (٧,١٤%) كان لديهن الأجسام المضادة نوع IgM ضد فيروس الحصبة الألمانية و ١٠ (٤,٧٦%) ضد فيروس HSV II بالإضافة ١٢ (٥,٧%) و ٧ (٣,٣%) لديهن الأجسام المضادة نوع IgG ضد . على التوالي APS, ACL

وان ٧ من النساء المجهضات أظهرت نتائج مشتركة مع الأجسام المناعية المضادة نوع IgM ضد الفيروس المضخم للخلايا خمسة منهم مع الأجسام المناعية المضادة نوع IgM ضد طفيلي المقوسات الكونيدية واثنان منهم مع الأجسام المضادة نوع IgM ضد فيروس الحصبة الألمانية.

كما أشارت الدراسة إن نسبة النساء المجهضات لمرة واحدة (٧١,٦%) أعلى من النساء المجهضات لمرة ثلث وثلث وان نسبة النساء المجهضات خلال الثلث الأول من الحمل كانت أعلى من النساء المجهضات خلال الثلث الثاني والثالث مع فرق معنوي عالي ($P < 0.001$)

Introduction

Pregnancy loss has been attributed to several factors involved in human reproduction. Genetic and uterine abnormalities, endocrine and immunological dysfunctions, infectious agents, environmental pollutants, psychogenetic factors and endometriosis are most important causes [1]

At all any severe maternal infection which leads to bacteraemia or viraemia can cause miscarriage. Consideration of the timing of the miscarriage is important, as different causes of miscarriage tend to manifest at different periods of gestation [2]

Recurrent pregnancy wastage due to maternal infections transmissible in utero at various stage of gestation can be caused by a wide array of organisms which include the TORCH complex (*Toxoplasma gondii*, *Rubella* virus, Cytomegalovirus, Herpes simplex virus) [3-6] and other agents like Coxsackie virus, *Ureaplasma urealyticum* [7], *Mycoplasma hominis* [8], *Chlamydia trachomatis* [9] *Treponema pallidum*, *Niesseria gonorrhoeae*, HIV etc [10].

These entire infectious agents induce a shift of immune response during pregnancy from Th2 to Th1 and apoptosis which can be observed clinically as an abortion process [11].

Other abnormalities including APS and ACL antibodies [12]. These antibodies are directed against anionic phospholipids or protein phospholipids complexes as in antiphospholipid syndrome (characterized by thrombosis, placental dysfunction, fetal death and the present of circulating antiphospholipid antibodies)[13]. History of recurrent pregnancy loss necessitating testing for antiphospholipid (APL) including (ACL and APS) antibodies [14] Even with implantation failure by

miscarriage, (APL) antibodies have been detected [15-16].

in Baghdad and Thiqr found that the high prevalence infection among aborted women was *T. gondii* 19.17%, 40% respectively [17].

Aim of this Study

Determination the prevalence of anticardiolipin (ACL), antiphosphatidylserine(APS) antibody and TORCH infections among aborted Women in Baghdad.

Materials and Methods

A total of 210 sera samples were collected from women with history of one or more consecutive unexplained repeated abortion during the pregnancy attending the Obstetrics and Gynecology department of Baghdad Teaching Hospital in medial city , AL-habibia Hospita and different private clinics in Baghdad between October 2012 to February 2013 in addition 45 sera samples from women with successful pregnancy as control group were the subject of this study.

All serum samples were screened for the presence IgM and IgG antibodies against *Toxoplasma gondii* ,Cytomegalovirus, *Herpes simplex virus* II, *Rubella virus*, anticardiolipin (ACL) and antiphosphatidylserine(APS) by using Enzyme-linked Immunosorbent assay (ELISA) according to the instruction for Biokit Diagnostics Company, Spain and all the samples were showing optical density above cut off value considered positive.

Results and Discussion

Abortive women were subdivided into three groups according to number of miscarriages (1, 2 and 3 miscarriages) the rate of one miscarriage in abortive women was 71.6% (86/120) higher than two and three miscarriages 25% (30/120), 3.4%

(4/120) respectively with significant difference ($P<0.05$) (Figure 1).

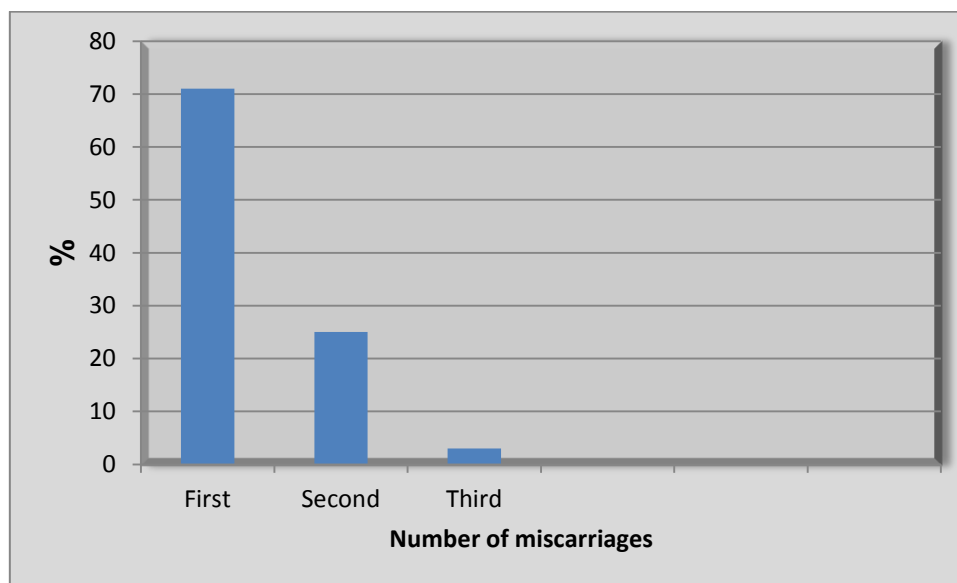


Figure 1 Distribution of 120 aborted women with seropositivity of ACL, APS, CMV, HSV II, Rubella virus and Toxoplasma gondii according to number of miscarriages

In this study , out of 210 aborted women in Baghdad a total of 44 (21%) were positive for IgM CMV *Toxoplasma gondii* IgM positive were 32 (15.23%), *Rubella* IgM positive were 15 (7.14%) and 10 (4.76%) were positive for *HSV II* IgM and the higher prevalence in IgM was in CMV 44 (21%) and the minimal was *HSV II* virus 10 (4.76%).

Also 12(5.7%) from 210 aborted Women involved in this study were positive for ACL IgG antibodies and negative for APS antibodies and 7(3.3%) women were positive for APS antibodies So either ACL or APS antibodies were found in 19 (9%) (19/210) of women with recurrent abortion and these antibodies were negative for control group (Table 1).

Our results were consistent with the other studies that 8-42% of recurrent abortion is due to ACL antibodies. [18] Also in this study our findings indicated that 3.3% of women with repeated abortion were positive for APS. The significant percentage of positive ACL has been noted in the

sera of women who had repeated pregnancy loss of unknown etiology. The IgG ACL was mentioned to be more significant than IgM in causing repeated abortion [19].

positive correlation between high serum ACA(anticardiolipin antibody) and pregnancy loss has been noted in several heightened immune mechanism, These antibodies themselves do not cause miscarriage but can specifically damage the inner wall of the blood vessel and cause blood clots in the placenta that block or slow down the baby's blood supply, causing growth to slow or the baby to die and miscarriage due to this problem can occur at any time in the first trimester and rarely past the first trimester with 10 to 15% of recurring miscarriages are caused by these antibodies [20, 21].

Also It has been shown also that Kuwaiti women with elevated serum titers of antibody against cardiolipin possess significantly higher levels of activated T cells and pathogenic B cells in the peripheral blood, suggesting a fundamental

predisposition to immune-mediated rejection of the fetus by these patients [22]. In addition high serum levels of anticardiolipin antibodies (ACA) are observed in peripheral blood of RSA-afflicted women; those with medium to high ACA have significantly lower pregnancy rates than their counterparts without ACA [23]. Moreover, supporting evidence to that Spontaneously aborting women elevated antiphospholipid antibodies often exhibit inadequate suppression of immune reactivity characterized by alterations in a number of immune parameters including increases in the percentage, absolute numbers and activity of peripheral blood NK cells [24] and NK-T cells (CD3 + CD16 + CD56+) and activated T cells were observed to be elevated in peripheral blood of high ACA, but not in normal ACA, patients relative to levels observed in healthy control subjects.

Primary CMV infection in pregnancy has a higher incidence of symptomatic congenital infection and fetal loss. This infection, being asymptomatic in adults, is difficult to diagnose clinically. Demonstration of IgM antibodies is indicative of primary infection. The present study shows seropositive rate of 21% for CMV IgM and this result agree with result has been reported previously.¹⁰ The need of serological evaluation of CMV specific IgM during pregnancy has been supported by various investigators [25,26]. Relationship between abortion and infection by cytomegalovirus and herpes simplex virus 1 and 2 was noted in 21.1% and

30.2% of women presented, respectively [27]

In the present study, *Toxoplasma gondii*, which is a known etiological agent in recurrent pregnancy loss was found in 15.23% aborted women in Iraq, a similar result was obtained by Abbas [28] showed that 21.5% of women with first abortion have positive only IgM by ELISA test. Al-Fertosi [29] and Salman [30] showed that 19.17% of women with single or repeated abortion by using ELISA test.

In addition, in Baghdad, Juma and Salman [31] found that the infection of *T. gondii* in women was 19.17%. In Tikrit, Al-Doorri [32] showed the presence of infection of about 49 % also in a study carried out in Basrah [33] the prevalence of Toxoplasmosis had been shown to be 41.1 to 52.1%, whereas a previous study by Al-Hamdani and Mahdi [34] showed low rate of 18.5% of Toxoplasma antibodies in Basrah population. In Duhok, North of Iraq [35] found low Toxoplasma infections of about 0.97%.

In Sulaimania, Karem [36] found out that by using ELISA, the seropositivity was 32.6% in women.

Seropositivity rate of HSV IgM among aborted women in this study was 4.76%. HSV in asymptomatic women with recurrent infection during pregnancy was found to be 0.6-3% previously and one of suggestion is that these infectious agents may be induce a shift of immune response during pregnancy from Th2 to Th1 and consequently rejection with an abortion process [37, 38].

Table 1 Results of ELISA in 210 women with spontaneous abortion and 45 women with successful pregnancy

Serological analysis	Ig	Aborted women		women with successful pregnancy	
		N= 210	%	N= 45	%
Rubella	IgM	15	7.14	Negative	0
	IgG	18	8.57	Negative	0
Toxoplasma gondii	IgM	32	15.23	Negative	0
	IgG	46	22	3	6.66
CMV	IgM	44	21	Negative	0
	IgG	61	29	8	17.77
HSV II	IgM	10	4.76	Negative	0
	IgG	10	4.76	Negative	0
Anticardiolipin (ACL)	IgM	Negative	0	Negative	0
	IgG	12	5.7	Negative	0
Antiphosphatidylserine (APS)	IgM	Negative	0	Negative	0
	IgG	7	3.3	Negative	0
56 aborted women with Unknown etiology	IgM	Negative	0		
	IgG	Negative	0		

Mixed infection were noted in 7 out of 44 patients(18.2%) in association with CMV IgM antibodies in this study. Out of 7 patients of mixed infection 5 were with

Toxoplasma gondii IgM , two with Rubella IgM and one mixed infection of *Toxoplasma gondii* IgM with Rubella IgM (Figure 2).

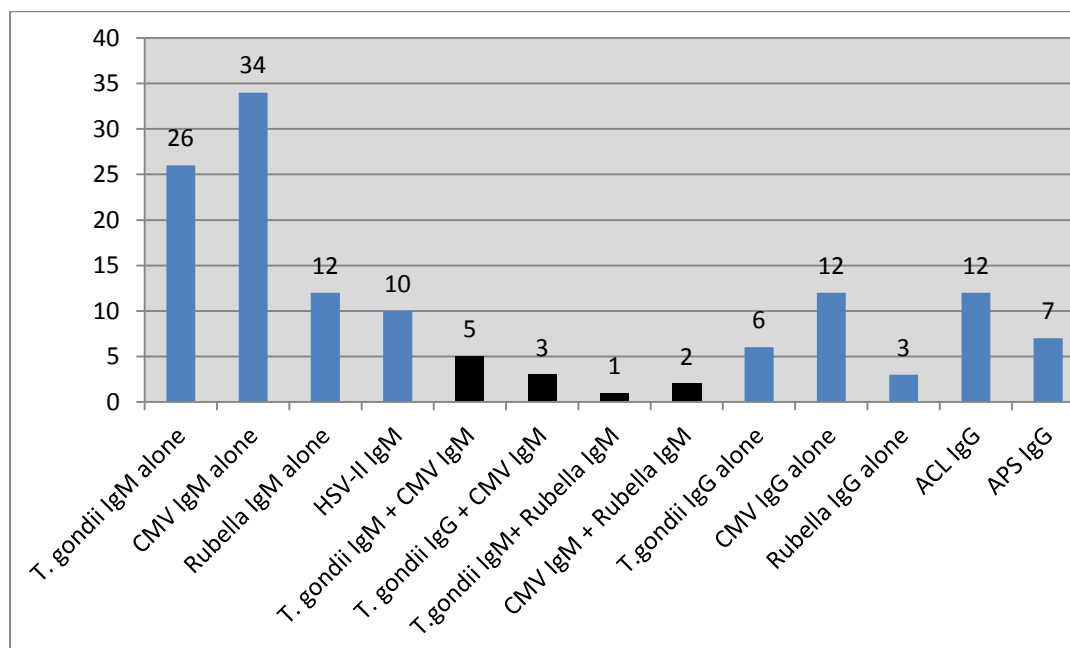


Figure 2 Distribution of aborted women according to single or mixed infection

Seroprevalence of *Toxoplasma gondii* IgG in combination with CMV IgM was 3(6.8%) out of total 44 samples showed positive CMV IgM. While Seroprevalence of IgG alone was higher 34(16.19%) in cytomegalovirus and lower in Rubella virus 3(1.42%) as well as IgG combination prevalence was noticed between *Toxoplasma gondii* and cytomegalovirus 5(2.38%) and didn't record result in Rubella virus and HSV-II with the other, total percentage was 38(40%).

The results pointed out that the seropositivity rate for CMV IgM, *T. gondii* IgM, Rubella IgM, HSV II IgM, ACL IgG and APS IgG were higher in first trimester abortion when compared with second and third trimesters abortion with highly significant difference ($P < 0.001$) (Table 3).

Mixed infection were observed of CMV IgM antibodies with

Toxoplasma gondii IgM in five patients and with Rubella IgM in two patients on other side one mixed infection of *Toxoplasma* IgM with Rubella IgM. Similar observation of mixed infection has been made earlier[39].

Moreover, The results pointed out that the seropositivity rate for CMV IgM, *T. gondii* IgM, Rubella IgM, HSV II IgM, ACL IgG and APS IgG were higher in first trimester abortion when compared with second and third trimesters abortion with highly significant difference ($P < 0.05$). To interpret that some maternal infections, especially during the early gestation, can result in fetal loss or malformations because the ability of the fetus to resist infectious organisms is limited and the fetal immune system is unable to prevent the dissemination of infectious organisms to various tissues [40].

Table 3 Distribution of 120 aborted women with seropositivity of ACL, APS, CMV, HSV II, *Rubella virus* and *Toxoplasma gondii* according to gestational age

Seropositivity	Gestational age Trimesters	N	%	Sig. between 1st and 2nd trimesters	
				P value	Statistical analysis
CMV IgM	1st	39	18.57	0.001	Highly significant
	2nd	5	2.38		
	Third	0	0		
T. gondii IgM	1st	23	11	0.05	significant
	2nd	9	4.28		
	Third	0	0		
Rubella IgM	1st	10	4.76	0.05	significant
	2nd	2	1		
	Third	0	0		
HSV II IgM	1st	10	4.76	0.001	Highly significant
	2nd	0	0		
	Third	0	0		
ACL IgG	1st	12	5.70	0.001	Highly significant
	2nd	0	0		
	Third	0	0		
APS IgG	1st	7	3.33	0.001	Highly significant
	2nd	0	0		
	Third	0	0		
Unknown etiology	1st			0.05	significant
	2nd				
	Third				

1st= first trimester abortion 2nd=second trimester abortion

Statistical analysis

In current our study we used statistical program SPSS version (20) to study the significance between tests in each type of antibody IgM and IgG, we used (t – test) after detection normal distribution to the data and appropriate $P < 0.05$ consider significant [41].

Conclusion

this study confirmed the significant association of infectious agents (*CMV Rubella*, *HSV II* and *Toxoplasma gondii*,) and APL antibodies such as ACL and APS with abortions with higher prevalence of CMV as major cause of abortions in Baghdad

Recommendation

Serological detection for each of *Toxoplasma gondii*, *Cytomegalovirus*, *Herpes simplex virus II*, *Rubella virus*, anticardiolipin (ACL) and antiphosphatidylserine (APS) by ELISA should be done for each women prepare to pregnancy.

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